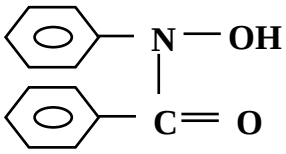
BINDING AFFINITY AND MOLECULAR MODELING ON INTERACTIONS OF HYDROXAMIC ACIDS WITH DNA

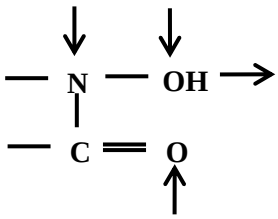
INTRODUCTION

Hydroxamic acids show a wide spectrum of biological activity as reported from this and other laboratories (1-10). N-Phenylbenzohydroxamic acid (PBHA) first introduced by Bamberger in 1919 (11), is a parent compound of N-arylhydroxamic acid's series.



PBHA

Introduction of various groups, viz. halogen, ethyl, methyl, methoxy or nitro etc. in the upper or/and lower phenyl ring gives a series of compounds. Hydroxamic acid functional group, a characteristic feature of these molecules contains, both Hydrogen-Bond Donor (HBD) and Hydrogen-Bond Acceptor(HBA) sites,



Which constitute an essential pharmacophoric structural part of these molecules.

Hydroxamic acids are neutral polyfuntional compounds and in life science Hydrogen-Bond interactions are the most important solute- solvent interactions for such molecules. Possibilities of forming Hydrogen –Bonds in structural part of the molecule also provide the binding sites for RECEPTORS. These are weak bonds easily form and break. Therefore, a molecule after exerting its effect, can easily leave the receptor . Besides, these molecules are " Drug Like Molecules" as they follow the "Lipinski Rule of 5" which states that a compound would be a drug like if it fulfil the following set of properties.

- **Molecular weight ≤ 500**
- **Lipophilicity ≤ 5**
- **HBD Sites ≤ 5**
- **HBA Sites ≤ 2×5**

In continuation of work carried out in this laboratory (12-18) on "QSAR and Drug Designing for Anti-tumor/Anticancer Activity", binding affinity of 5-10 hydroxamic acids with ctDNA will be studied by following methods (1) UV-Vis absorption spectroscopy (2) Fluorescence spectroscopy (3) Fluorescence microscopy(4) Viscometry (5) Gel electrophoresis and docking.

DNA is an important target for several drugs, and molecular docking and is an important approach to evaluate the interactions between potential ligands and the target (19-20). The docking studies are totally lacking in case of N-arylhydroxamic acids with DNA although these molecules have been recognised as anti-tumour/anti-cancer agents (21). Holt et al (22) showed that Auto dock and surflux can accurately reproduce the crystal structure of several ligands bound to DNA (minor groove binders and intercalators) within a resolution of approximately 2 Å. This solve the problem that those docking programs which are validated for proteins are also applicable in ligand-DNA docking. This knowledge is useful to understand the potential of automated docking programs for virtual screening of DNA binding agents.

In general interaction of small molecules with DNA occurs in two different ways (23),

- Intercalation between base pairs
- Groove recognition

The major groove offers, more HBD and HBA sites. It also provides a much larger and shallow binding pocket for small molecular binding than the smaller groove , while the major groove is the preferred binding site for proteins and peptides (24-25).

Among the main DNA binding modes , intercalation is the most common way through which small aromatic molecules recognise DNA. It is considered as an induced-fit mechanism. The sequence-specific ligands show preference for some sequences and it is important to observe that sequence selectivity which can arise in three ways,

I) Direct readout of hydrogen-bonding pattern,

II) Indirect readout of sequence flexibility.

III) Combination of both.

Therefore, docking method that do not take into account DNA flexibility are sensitive to (I) only. Thus, as a primary approach to docking studies of DNA-interacting agents explains regarding the binding mode more correctly than investigating the existence of preferential binding sites. The docking protocol may further be used to predict the binding modes.

In contribution of studies on "QSAR and Drug Designing for Anti-Tumor/Anti-cancer Activity" in our laboratory, the binding affinity of a series of hydroxamic acids with DNA will be studied to test the docking-protocol with these molecules that bind covalently to DNA and to compare the results with self docking will be the target of investigation in the present studies.

A BRIEF REVIEW OF THE WORK ALREADY DONE IN THIS FIELD

DNA-binding affinity for some molecules of this series have been reported from this laboratory only (26), where's, the field of molecular docking of totally remains untouched for the hydroxamic acids to be studied in the present investigation.

The studies on molecular docking are never performed on the derivatives of PBHA.

OBJECTIVE

N-Arylhydroxamic acids have been reported as Anti-Tumor/Anti-cancer agents from this laboratory (27). Further, to know how these molecules bind to DNA, the present investigation is undertaken.

PROPOSED METHODOLOGY

The work will be distributed in the following five chapters.

CHAPTER I: INTRODUCTION

In this chapter, introduction of hydroxamic acids, DNA and molecular docking will be described with recent literature survey data.

CHAPTER II: SYNTHESIS OF HYDROXAMIC ACIDS

N-Arylhydroxamic acids and their derivatives will be synthesized in the laboratory following the steps reported in literature (28-29).

SYNTHESIS OF HYDROXAMIC ACIDS

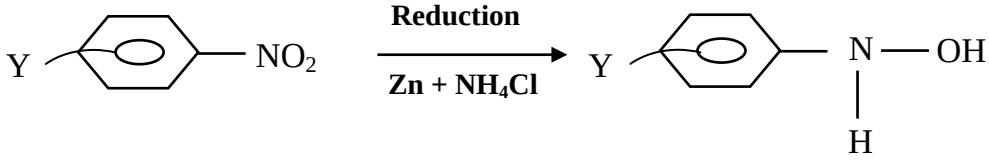
Synthesis of hydroxamic acids is a three step process.

Step I : Preparation of Acid Chloride

Acid chloride will be prepared by the action of thionyl chloride on corresponding carboxylic acids.

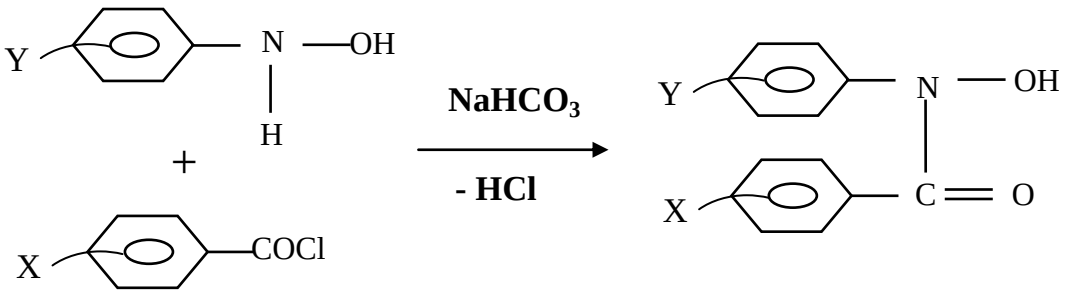
Step II : Preparation of Hydroxylamines

Hydroxylamine will be prepared by the reduction of corresponding nitro compounds in neutral medium.



Step III : Synthesis of Hydroxamic Acids

Hydroxamic acids will be synthesized by coupling the hydroxyl amine with acid chloride in presence of sodium bicarbonate, in ether medium.



where, X and Y are different substituent's at various position in upper or/and lower phenyl rings.

Following hydroxamic acids will be synthesized for the present investigation,

- **N-1-Naphthyl-N-Lauro Hydroxamic Acid**
- **N-1-Naphthyl-Caporic Hydroxamic Acid**
- **N-1-Naphthyl-Myristo Hydroxamic Acid**
- **N-p-Tolyl-Undecane Hydroxamic Acid**
- **N-p-Tolyl-Stearo Hydroxamic Acid**
- **N-Phenyl-Stearo Hydroxamic Acid**

CHAPTER III: HYDROXAMIC ACIDS-DNA BINDING PARAMETERS

The molecular interactions between drugs and DNA have great importance to study their biological activity and become an active research area in recent years. DNA is the material of inheritance and controls the structure and functional activities of cells. Recently, there has been tremendous interest in studies related to the interaction of small molecules with nucleic acid because of their relevance in the development of new reagents for biotechnology and medicine . These studies are also important to understand toxicity and molecular mechanism of the drug action to design a specific DNA-targeted molecule.

The possible interaction ways between small molecules and DNA generally follow three models,

[i] Electrostatic binding between cation species and negative charge DNA phosphate.

[ii] Groove binding in which molecule bounded in groove of DNA involving hydrogen bonding or Vander Waals interaction force.

[iii] Intercalative binding involves between drug and base pairs. DNA binding is the critical step for determing biological activity.

In present chapter UV absorption, fluorescence, viscosity, DNA melting, thermodynamic and photocleavage activity will be used to explore the interaction between hydroxamic acids and DNA. The results obtained will be compared with the software based docking binding parameters.

HYDROXAMIC ACIDS-DNA BINDING PARAMETERS will be evaluated by following methods.

1. **UV ABSORPTION METHOD** UV- absorption is one of the method to investigate the binding of a molecule with DNA. The UV-absorption spectroscopy experiments will be conducted by adding a fix amount of DNA solution to its sample of hydroxamic acids (HA) at different concentrations(C). The pH around 7.0 will be adjusted using buffer. The absorption spectra will be recorded in the absence and presence of DNA.

The binding constants will be calculated following the equation (30-31),

$$\frac{\mathbf{DNA}}{\varepsilon_a - \varepsilon_t} = \frac{\mathbf{DNA}}{\varepsilon_b - \varepsilon_t} + \frac{1}{K_b(\varepsilon_b - \varepsilon_t)} \qquad \textbf{[1]}$$

where,

DNA = Concentration of DNA.

$\varepsilon_{a,}$ = Apparent extinction coefficients. ($A_{obs}/[M]$).

ε_t = Extinction coefficients of ligand.

ε_t = Extinction coefficients of ligand-DNA complex in bound form.

K_b = Binding constant.

2. THERMODYNAMIC METHOD

The acting forces between small molecules and DNA include hydrogen bond, Vander waals forces, hydrophobic interaction and electrostatic forces. According to Ross, binding forces

are estimated by ΔH and ΔS, when $\Delta H<0$ or $\Delta H\approx 0$, $\Delta S>0$, the mainly acting force is electrostatic, van der Waals or hydrogen bond and $\Delta H>0$, $\Delta S>0$, shows hydrophobic as main force [32-33].

The values of ΔH and ΔS will be obtained from Van't Hoff equation,

$$\mathbf{Log\ K = \Delta H/2.303RT + \Delta S/2.303R} \qquad \mathbf{[2]}$$

$$\mathbf{\Delta G = \Delta H - T\,\Delta S} \qquad \mathbf{[3]}$$

3. FLUORESCENCE METHOD

A quantitative estimation of quenching experiments in terms of the intrinsic binding constant calculation will be obtained from Stern Volmer equation,

$$\mathbf{\log (F_o\text{-}F)/F = \log K_b + n \log[Q]} \qquad \mathbf{[4]}$$

where,

F_0 = Fluorescence intensities in absence of quencher.

F = Fluorescence intensities in presence of quencher.

Ks = Stern-Volmer quenching constant.

Q = Concentration of quenchers.

4. COMPETITIVE BINDING WITH ETHIDIUM BROMIDE

Further support for the mode of binding between hydroxamic acids and DNA is given through the competitive experiment. Here ethidium bromide (EB) has been employed in the examination of the reaction, as EB presumably binds initially to DNA by intercalation and the fluorescence of EB increases after binding with DNA. Like EB, if hydroxamic acids intercalate into the helix of DNA, it would compete with EB for the intercalation sites, and lead to a significant decrease in the fluorescence intensity of the DNA–EB complex.

5. DNA MELTING METHOD

The double-helical structure of DNA is remarkably stable due to hydrogen bonding and base stacking interaction. Upon increasing the temperature the dissociation of helix to single strand increases. The melting temperature T_m, which defines as the temperature where half of total base pair is unbounded, will be determined as transition midpoint of melting curve (34). The change in T_m is observed due to interaction of hydroxamic acids to DNA.

6. BY VISCOSITY MEASUREMENTS

Viscosity describes a fluid's internal resistance to flow and may be thought of as a measure of fluid friction. This resistance becomes apparent when a layer of fluid is made to move in relation to another layer. Viscosity is a principal parameter when any flow measurements of fluids, such as liquids, semi-solids, gases and even solids are made. The greater the friction, the greater the amount of force required to cause this movement, which is called shear. The velocity gradient, dv/dx, is a measure of the change in speed at which the intermediate layers move with respect to each other. It describes the shearing the liquid experiences and is thus called shear rate. The viscosity of DNA solution increases due to the binding of drug molecules. Viscosity experiment is an effective tool to determine the binding mode of small molecules and DNA. The relative specific viscosity will be obtained by Eq. (35-36),

$$\eta_r = \sqrt[3]{\frac{\eta}{\eta_0}} \qquad [5]$$

where,

η_0 = Specific viscosity contributions of DNA in the absence of the complex,

η = Specific viscosity contributions of DNA in the presence of the complex.

7. BY PHOTOCLEAVAGE ACTIVITY METHOD

For the gel electrophoresis experiment, pBR322 super coiled plasmid DNA will be treated with the hydroxamic acids in Tris-acetate buffer of pH 7.4 at room temperature. The samples

will be analyzed by electrophoresis for 1 hour at 100V in Tris-acetate buffer containing 1% agarose gel.

CHAPTER IV: MOLECULAR MODELLING

I. HEX DOCKING WITH DNA

All docking studies will be carried out on an Intel i5 4GB RAM,3.2 GHZ station ,500 GB memory with Linux operating system using Hex docking. The B-DNA duplex receptor will be obtained from protein data bank. The Q-finder will be used to find the sites in selected model. A number of 150 cycles of calculation will be used in order to get a final binding position as accurate as possible. Hex uses Spherical Polar Fourier (SPF) correlations to accelerate the calculations and its one of the few docking programs. The parameters used for the docking process via Hex docking Software are,

- Correlation type – Shape and ESP
- FFT Mode – 3D
- Grid Dimension – 0.6
- Receptor range – 180
- Ligand Range – 180
- Twist range – 360
- Distance Range – 40

II. COMPARATIVE MOLECULAR FIELD ANALYSIS (COMFA)

Most drugs that are now a days used in human therapy interact with certain macromolecular biological targets, e.g., with enzymes , receptors , ion channels , and transporters, in some cases even with deoxyribonucleic acid (37-38). Most drug actions result from the non covalent association of a small ligand (the drug) to a specific binding site at the macromolecule. Thus, a precondition for the biological activity of a drug is its high affinity to this binding site. In addition to their affinity to a certain binding site, they have different intrinsic activities, i.e., different abilities to produce the agonist-mediated biological effect. Presently, the most reasonable hypothesis is that receptor agonists stabilize the ‘active’ conformation of a receptor, whereas antagonists stabilize its ‘inactive’ conformation (39-40). The building blocks of all biological macromolecules belong to one group of optically active enantiomers. Thus, all drug receptors (the term ‘receptor’ is most often generally used for any

biological macromolecule which is the binding partner of a drug) are themselves enantiomers, i.e., they possess asymmetrical geometries. Therefore it is not surprising that enantiomers of chiral drugs differ in their biological activities, a fact that is not adequately considered in most quantitative structure activity relationships (QSAR) studies. Classical QSAR correlates biological activities of drugs with physicochemical properties or indicator variables which encode certain structural features. In addition to lipophilicity, polarizability, and electronic properties, steric parameters are also frequently used to describe the different size of substituents. In some cases, indicator variables have been attributed to differentiate racemates and active enantiomers. QSAR analyses consider neither the 3D structures of drugs nor their chirality.

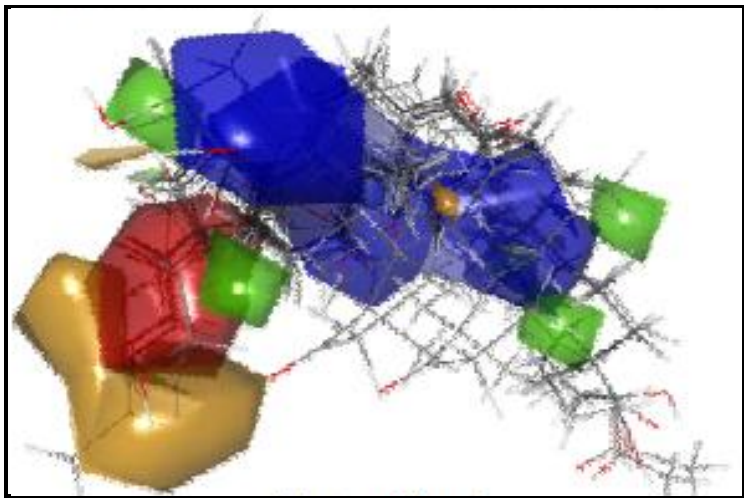


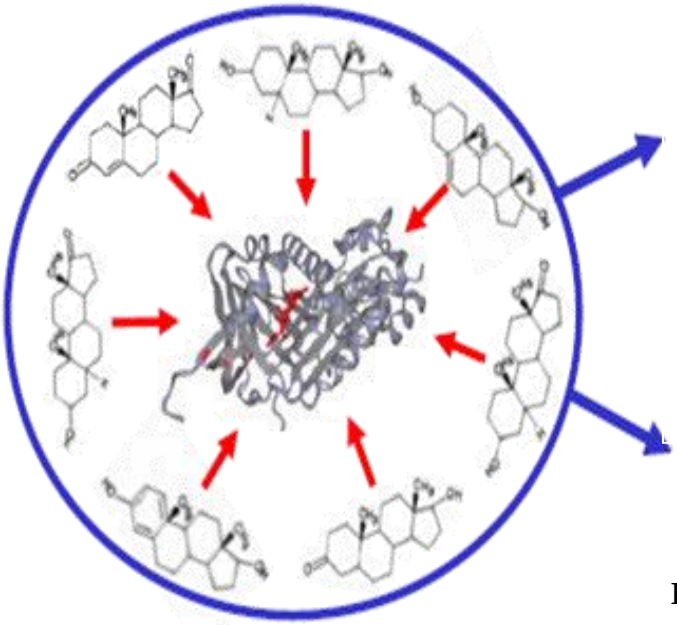
Fig. 1

The 3D-QSAR, comparative molecular field analysis (CoMFA) method, proposed by Cramer et al. in 1988, is extensively used, in the present practice of drug discovery (41). One of the advantages of CoMFA is its ability to predict the biological activity of molecules by deriving a relation between steric/electrostatic properties and biological activities in the form of contour maps. Comparative molecular field analysis calculates steric fields using Lennard–Jones potential and electrostatic fields using a Coulombic potential. In particular, both of the potential functions are very steep near the van der Waals surface of the molecule, causing rapid changes in the surface descriptions and requiring the use of cut-off values so that calculations are not done within the molecular surface. In addition, a scalar factor is applied to the steric field, so that both fields can be used in the same partial least-square (PLS) analysis. Changes in the orientation of the superimposed molecular set, relative to the calculation grid, can cause significant change in the CoMFA results, again probably because

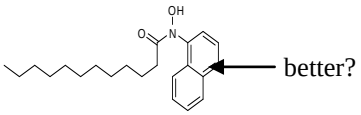
of strict cut-off values. So,the alignment rules are one of the most sensitive input areas for COMFA studies.

Given : Set of ligands with known binding affinities to a receptor binding site

Wanted : To find a better ligand (higher affinity)



Lead optimisation/design : Which parts have to be modified and how?



Binding affinity prediction : How well does another ligand bind ?

Log(1/k)=???

→ **CoMFA can help**

Fig.2

APPLICATIONS

- To develop quantitative structure-activity relationships.
- To predict the properties and activities of untested molecules.
- To compare different QSAR models statistically and visually and to optimize the properties of a lead compound.
- To validate models of receptor binding sites.
- To generate hypotheses about the characteristics of a receptor binding site.

- To prioritize compounds for synthesis or screening.
- To determine key structural requirements for high affinity receptor ligands.

EXPECTED OUT COME OF THE PROPOSED WORK

1. This work will explore the binding interaction of N-aryl hydroxamic acids with DNA and will lead to the understanding of the dna binding parameters ,thermodynamically and spectroscopically.

2.This investigation will throw light in the understanding of anticancer, antitumour, anti fungal and other medicinal properties of N-aryl hydroxamic acids at the molecular level.

3. Molecular modelling will help in the screening of the hydroxamic acids as drug and further co-relation between the experimental and the theoretical value will be established.

4.The knowledge gained from this study will be helpful to understand the pharmacological effects of N-aryl hydroxamic acids and in the design of the structure of new and efficient drug molecules .

REFERENCES

1. Rho, H. S.; Baek, H. S.; Ahn, S. M.; Yoo, J. W.; Kim, D. H.; Kim , H. G.; Bull. Korean Chem. Soc., **30** (2009).
2. Seo, S. K.; Jin, H. O; Lee, H. C; Woo, S. H.; E.-S. Kim, E.S.; Yoo, D. H. ; Lee, S. J.; Sungkwan, A.; Rhee, C.H.; Hong, S. I.; Choe, T. B.; Park., I. C.; Mol Pharmacol , **73**, 1005 (2008).
3. Balliet, R. M.; Chen, G.; Gallagher, C. J. ; Dellinger, R. W. ; Sun, D.; Lazarus,P.; Cancer Research, **69**, 2981 (2009).
4. Kim, S. A.; Jin, Y. L. ; Kim, H. S.; Arch Pharm Res., **32** , 15 (2009).
5. Nguyen, A.; Su, L.; Campbell, B.; Poulin, N. M.; Nielsen, T. O.; Sarcoma volume (2009).

6. Butovich, I. A.; Lukyanova, S. M.; J. Lipid Res., **49**, 1284 (2008).
7. Lawrence, P.; Tardibono, Jr.; Miller, M. J.; Org. Lett., **11** , 1575 (2009).
8. Saban, N.; Bujak, M.; Cancer Chemother Pharmacol., **64**, 213 (2009).
9. Julija, M. S.; Ita, S. ; Lorena, H.; Blazenka, J. ; Acta Pharm ; **58** , 231 (2008).
10. Lossen, H.; Ann. Chem., **150**, 314 (1869).
11. Bamberger, E.; Ber., **52**, 116 (1919).
12. Kumar, A.; Rajwade, R. P.; Pandey, B. N.; Pande , R.; Mishra, K. P; Journal of cell and Tissue Research,**7**, 943 (2007) .
13. Rajwade, R. P.; Pande, R.; Mishra, K. P.; Kumar, A.; Pandey, B. N.; Medicinal Chemistry, Bentham Science, **4** , 237 (2008).
14. Rajwade, R. P.; Pande, R.; Analytica Chimica Acta, **630**, 205 (2008).
15. Pande. R.; Tondon, S. G.; J. Chem. Engg. Data, **24**, 72 (1979).
16. Rajwade, R.P.; Pande, R.; Ana. Chem. Acta., **630**, 205 (2008).
17. Tiwari, V.; Pande, R.; J. Mol. Liq., **128**, 178 (2006).
18. Yadav, S.S.; Pande, R.; Khare, D.; Tripathi, M.; J. Chem. Thermodynamics, **54**, 76 (2012).
19. Kitchen, D. B.; Decornez, H.; Furr, J. R.; Bajorath;Nat. ReV. Drug DiscoVery, **3**, 935–949 (2004).
20. Moitessier, N.; Englebienne, P.; Lee, D.; Lawandi, J.; Corbeil, C. R.;J. Pharmacol.,**153**, S7–S26 (2008).
21. Rajwade, R. P.; Kumar, A.; Pandey, B. N.; Pande , R.; Mishra, K. P.;J.QSAR comb. Sci.,**28**,1500-1508 (2009).
22. Holt, P. A.; Chaires, J. B.; Trent, J. O.; J. Chem. Inf. Model., ***48***, 1602–1615 (2008).
23. Hannon, M. J.; Chem. Soc. ReV. , **36,** 280–295(2007).
24. Tse, W. C.; Boger, D. L.; Chem. Biol., **11**, 1607– 1617 (2004).
25. Shen, H.Y.; Shao X.L. ; Dongpan.S. ;Int. J. Electro Chem.. Sci . **6,** 532-547 (2011).
26. Khare, D.; Pande, R., Der Pharma Chemica, **4** , 66 (2012).
27. Kumar, A.; Rajwade, R. P.; Pandey, B. N.; Pande, R.; Mishra, K. P., Journal of cell and Tissue Research. **7**, 943 (2007).

28. Gupta, V.K.; Tandon, S.G.; J. of Chem. Engg. Data, **17**, 248 (1972).
29. Pande, R.; Tondon, S.G.; J. Chem. Engg. Data, **24**, 72 (1979).
30. Motlagh, M.K.; Noroozifar, M.; Khmmarnia, S.; Spectrochimica Acta Part A, **78**, 389 (2011).
31. Mudasira; Wahyunia, E.T.; Tjahjonob, D.H.; Yoshiokac, N.; Inouec, H.; Spectrochimica Acta *Part A*, **77**, 528 (2010).
32. Barcelo, F.; Portugal, J.; FEBS Letters, **576,** 68 (2004).
33. Zhang, G.; Hu, X.; Pan, J.; Spectrochimica Acta Part A, **78**, 687 (2011).
34. Wolff, M. E., (ed.), 'Burger's Medicinal Chemistry', **Vol. I**:Principles and Practice, Wiley, New York, 5th edn., (1995).
35. Liu, J.G.; Ye, B.H.; Li, H.; Zhen, Q.X.; Ji, L.N.; Fu, Y.H.; J. Inorg. Biochem., **76**, 265 (1999).
36. Janjua, N.K.; Shaheen, A.; Yaqub, A.; Perveea, F.; Sabahat, S.; Mumta, M.; Jacob, C.; Ba, Motlagh, M.K.; Noroozifar, M.; Rigi, S.M.; Spectrochimica Acta Part A, **75**, 598 (2010).
37. C. G. Wermuth, (ed.), 'The Practice of Medicinal Chemistry',Academic Press, London, (1996).
38. Ohm, H. J. B.; Klebe, G.; Kubinyi, H.; 'Wirkstoffdesign',Spektrum Akademischer,Heidelberg, (1996).
39. Ramsden,C. A.; (ed.), 'Quantitative Drug Design', Vol. 4, of 'Comprehensive Medicinal Chemistry', eds. C. Hansch, P. G. Sammes, and J. B. Taylor, Pergamon, Oxford, 1990.
40. H. Kubinyi, 'QSAR: Hansch Analysis and Related Approaches',VCH, Weinheim, 1993.
41. Cramer, R.D.; Patterson, D.E.; Bunce, J.D.; J Am Chem Soc,**110**,5959–5967 (1988).

Signature of the Supervisor **Signature of the candidate**

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